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By GERTRUDE RODNEY

A DISSERTATION SUBMITTED IN
PARTIAL FULFILMENT OF THE REQUIREMENTS FOR
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THE OXIDATIVE DEAMINATION OF SOME STRUCTURALLY RELATED AMINOPROPIONIC ACIDS BY THE LIVER AND KIDNEY TISSUES OF THE RAT

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The original observations of Meyerhoff, Lohmann and Meier (1) and of Reinwein (2) on the decomposition of amino acids by surviving tissues have been fully confirmed and extended by a number of investigators (3-5). Krebs (6, 7) has made a particularly thorough study of this problem and has presented excellent evidence that liver and kidney tissues act upon amino acids by a process of oxidative deamination. The results of these studies thus support the older views of Neubauer (8) and Knoop (9).

It is an interesting fact, however, that although the animal organism, as a whole, is concerned chiefly with the metabolism of the naturally occurring amino acids of the *l* series,¹ the individual tissues, in *in vitro* experiments, exhibit a much greater deaminizing activity toward the unnatural *d*-amino acids. The physiological rôle of the *d*-amino acid deaminase is not clear. Krebs (11) has suggested that this enzyme may be a fragment of the deaminizing system of the intact tissue, in which case the results of the various experiments are of doubtful significance in metabolism. On the other hand, it must be recognized that the *d*-deaminase may function as an agent by means of which the organism resolves racemic mixtures of amino acids and thus produces the *l* forms. There is considerable evidence that the *d* forms of certain amino acids are readily utilized in the animal organism (12-14) and the recent findings of Braunstein and Kritsman (15), that amino acids are synthesized in the tissues by the intermolecular transfer of an amino group from an amino acid

¹ The nomenclature for the optical isomers proposed by Wohl and Freudenberg (10) is used throughout this paper.

to a keto acid, suggest that racemic mixtures of amino acids may arise as intermediates in the metabolic process.

In view of the fundamental importance of these questions to the whole problem of amino acid metabolism, a somewhat sharper definition of the specificity of the deaminizing system is desirable. The experiments reported here are concerned with the relationship of structural isomerism to the specificity of the *d*-deaminase of liver and kidney. A number of aminopropionic acids have been studied with respect to their oxidation and deamination by rat tissues and it has been shown that the enzymes in these tissues act only upon the α -amino groups of the compounds in this series.

EXPERIMENTAL

The accuracy of the experimental technique was checked by a large number of experiments with *dl*-alanine and *dl*-phenylalanine. These amino acids have been the substrates of choice in a number of investigations (3, 5, 16), and the results obtained here are in good agreement with those reported in the literature. It was found, however, that the respiration of the tissues alone was of such a magnitude that the effects of the added amino acids were obscured. Szent-Györgyi (17) has shown that tissue respiration is markedly suppressed by the presence of sodium arsenite which prevents further oxidation of α -keto acids. Krebs has used this reagent in experiments designed for the isolation of the products of the deamination of amino acids and has shown that its presence has no effect upon the deaminizing system. It has been considered expedient, therefore, to include arsenite in the suspension medium of every experiment. Under these conditions, it was demonstrated that the oxidation of the amino acids mentioned above took place at a fairly rapid rate, and with the kidney tissues the oxygen consumption and the ammonia production approached the theoretical values demanded by the assumption that the *d* isomer alone was oxidized. The respiration of the liver tissues in the presence of the amino acids was not as large as that observed with the kidney tissue nor was the deamination as extensive over a given period of time.

Amino Acids—The *d*-, *l*-, and *dl*-alanine and the *dl*-serine were commercial products. The β -alanine was prepared according to the directions of Clarke and Behr (18); *dl*- α , β -diaminopropionic

acid, by the method of Neuberg and Silberman (19); and *dl*-isoserine, by the ammonification of β -chlorolactic acid (20). All of the amino acids were recrystallized until the presence of ammonia could no longer be detected and until the analyses for total nitrogen and amino nitrogen agreed with the theoretical values.

Tissues—The tissues of young male rats, 125 to 150 gm. in weight and previously fasted for 24 hours, were used. The animal was stunned by a sharp blow upon the back of the head and the liver or the kidneys were removed as rapidly as possible and placed in ice-cold Ringer-phosphate solution. The slices were cut with a new razor blade, squared over cross-section paper, and placed in the various manometer vessels. The dry weight of the slices was usually between 5 and 8 mg.

Solutions—The buffered medium used throughout the investigation was a Ringer-phosphate solution of pH 7.4, prepared according to the directions of Krebs (6) with the exception that calcium chloride was not included in the mixture.

The stock solution of sodium arsenite was varied according to the volume of the medium to be used in a particular series of experiments. The salt was dissolved in the Ringer-phosphate medium in an amount such that the addition of 0.5 ml. of the solution to the medium in the manometer vessel produced a final concentration of 0.005 M.

The concentration of the amino acid was found to exert a rather marked effect upon the extent of deamination. The optimum concentration varied with the different amino acids. The general procedure adopted was to place in the side arm of the manometer vessel enough of the amino acid solution so that when this was mixed with the tissue and medium the final concentration was either 1 mg. per ml. or 0.005 M. The majority of the studies was made at these concentrations and the agreement in both instances was satisfactory.

Procedure—The constant volume Warburg-Barcroft apparatus was used. Seven respirometers were employed in each experiment, one of which served as a thermobarometer. The six experimental vessels thus permitted duplicate determinations with the tissue alone, with the tissue plus the particular amino acid under investigation, and with the tissue plus *dl*-alanine. The study of the latter amino acid was included as a control in every

experiment, since a certain amount of variation in the enzyme content of the tissues of different animals was observed, and, unless the oxygen uptakes with *dl*-alanine were satisfactory, the entire experiment was discarded.

The respirometers were filled with oxygen and were maintained at a temperature of 37°. The experiments were usually continued through a 6 hour period.

At the end of the respiration study, the vessels were opened and the tissues were removed, washed, and placed in tared crucibles to be dried and weighed. The washings were added to the solution in the vessel and this mixture was deproteinized with metaphosphoric acid. Aliquots of the filtrate were used for the ammonia determinations by the Holmes and Watchorn modification of the Sanford method (21).

Calculations—All of the respiration data are expressed in terms of the per cent of the theoretical oxygen uptake. These values were calculated in the following manner. The amounts of oxygen consumed by the tissue controls, expressed in cu. microliters, were divided by the dry weights of the respective tissues and the quotients were averaged. The value obtained was the average oxygen consumption per mg. of dry tissue and it has been assumed that this factor was constant for each of the tissue slices. The residual respiration for each of the experimental slices was then calculated by multiplying the dry weight of the tissue slice by the average oxygen uptake of the control tissues. Subtraction of this quantity from the total oxygen consumption gave the oxygen used for the deamination process. This absolute value, expressed in cu. microliters, was then compared directly with the calculated amount of oxygen which would have been required for the complete deamination of that quantity of the amino acid which was present at the beginning of the experiment. The data from the ammonia determinations were similarly treated.

It is believed that this use of absolute percentages, calculated from two types of analytical data, furnishes a superior basis for the comparison of the various experiments than does the expression of the data as rates. This is particularly true in the case of the ammonia in which the values were obtained at the end of the experiment and the rate of formation could not be calculated with any degree of accuracy.

DISCUSSION

In Tables I and II are presented data typical of the results obtained with a large number of slices of kidney and liver. In these experiments, the concentration of the amino acids was

TABLE I

Oxidative Deamination by Rat Kidney

The final concentration of the amino acids was 0.005 M. The theoretical oxygen uptake and ammonia values are calculated upon the basis that one optical isomer only is oxidized. The experiments were of 6 hours duration.

Amino acid	Oxygen consumption			Ammonia production		
	Theoretical	Observed		Theoretical	Observed	
	c.mm.	c.mm.	per cent theoretical	c.mm.	c.mm.	per cent theoretical
<i>dl</i> -Alanine	56	66	118	0.085	0.091	107
	56	58	104	0.085	0.081	95
<i>d</i> -Alanine	112	114	102	0.170	0.162	95
	112	88	79	0.170	0.111	65
<i>dl</i> -Alanine	56	64	114	0.085	0.093	109
<i>l</i> -Alanine	112	-1	-1	0.170	0.001	0
	112	-1	-1	0.170	0.001	0
<i>dl</i> -Alanine	56	63	113	0.085	0.087	102
	56	60	107	0.085	0.085	100
β -Alanine	112	-9	-8	0.170	-0.001	-1
	112	-2	-2	0.170	0.002	1
<i>dl</i> -Alanine	56	58	104	0.085	0.082	96
	56	64	113	0.085	0.101	118
<i>dl</i> - α , β -Diamino-propionic acid	56	19	34	0.085	0.027	32
	56	22	39	0.085	0.035	41
<i>dl</i> -Alanine	56	72	129	0.085	0.094	110
	56	73	131	0.085	0.099	116
<i>dl</i> -Serine	56	37	65	0.085	0.056	66
	56	38	68	0.085	0.048	56
<i>dl</i> -Alanine	56	46	81	0.085	0.078	92
	56	50	89	0.085	0.079	94
<i>dl</i> -Isoserine	56	-1	-1	0.085	-0.002	-2
	56	0	0	0.085	0.001	1

0.005 M, but essentially the same results were obtained when the concentrations were 1 mg. per ml. The agreement between the per cent of decomposition as determined by the oxygen consumption and that calculated from the ammonia analysis was quite

satisfactory. In general, the results obtained with the liver slices were more variable and of a lesser magnitude than were those with kidney.

The results with *d*- and *l*-alanine indicate that only the *d* form of this amino acid is attacked. In a number of the experiments with *dl*-alanine, however, values over 100 per cent were obtained, which indicate that the *l* isomer may be decomposed to a small

TABLE II

Oxidative Deamination by Rat Liver

The final concentration of the amino acids was 0.005 M. The theoretical oxygen uptake and ammonia values are calculated upon the basis that one optical isomer only is oxidized. The experiments were of 6 hours duration.

Amino acid	Oxygen consumption			Ammonia production		
	Theoretical	Observed		Theoretical	Observed	
	<i>c.mm.</i>	<i>c.mm.</i>	<i>per cent theoretical</i>	<i>c.mm.</i>	<i>c.mm.</i>	<i>per cent theoretical</i>
<i>dl</i> -Alanine	56	22	40	0.085	0.026	31
	56	16	29	0.085	0.022	26
β -Alanine	112	0	0	0.170	0.001	1
	112	1	1	0.170	0.002	1
<i>dl</i> -Alanine	56	27	49	0.085	0.046	52
<i>dl</i> - α , β -Diamino-	56	16	28	0.085	0.022	26
propionic acid	56	18	32	0.085	0.025	33
<i>dl</i> -Alanine	56	23	42	0.085	0.031	36
	56	19	34	0.085	0.022	26
<i>dl</i> -Serine	56	17	31	0.085	0.018	21
	56	16	28	0.085	0.015	18
<i>dl</i> -Alanine	56	17	31	0.085	0.023	27
	56	16	29	0.085	0.033	39
<i>dl</i> -Isoserine	56	-4	-7	0.085	-0.001	-1
	56	-2	-4	0.085	0.000	0

extent, when present as a component of the racemic mixture. These results are in agreement with those of Krebs who found that certain of the *dl*-amino acids consumed oxygen in excess of the theoretical quantity required for one isomer only.

In no instance could deamination of β -alanine be demonstrated. Krebs has reported similar findings with this amino acid, but Kisch (22) has found that the respiration of horse and ox tissues

was increased by the addition of β -alanine. The maximum value found in the present study was an increase in the respiration of 3 per cent, whereas the average increase was less than 1 per cent. Since these increases are within the limits of error of the methods, it is evident that β -alanine is not decomposed by rat tissues under the conditions of experimentation used here.

Essentially similar results were found with the *dl*-isoserine. In none of the experiments with this amino acid was increased

TABLE III

Summary of Oxygen Consumption and Ammonia Production

The concentration of the amino acid was 0.005 M in every experiment. All of the values are expressed in per cent of the theoretical.

Substrate	Tissue	No. of experiments	Oxygen consumption			Ammonia production		
			Maxi- mum	Mini- mum	Aver- age	Maxi- mum	Mini- mum	Aver- age
			per cent	per cent	per cent	per cent	per cent	per cent
<i>dl</i> -Alanine	Kidney	22	135	70	106	124	65	100
β -Alanine	"	4	2	-8	-2	2	-1	0
<i>dl</i> - α , β -Diamino- propionic acid	"	4	39	27	33	41	20	30
<i>dl</i> -Serine	"	4	68	35	53	66	40	53
<i>dl</i> -Isoserine	"	4	0	-3	-2	1	-2	-1
<i>dl</i> -Alanine	Liver	16	63	28	41	54	26	38
β -Alanine	"	4	3	-3	0	4	0	3
<i>dl</i> - α , β -Diamino- propionic acid	"	4	32	28	30	30	25	28
<i>dl</i> -Serine	"	4	31	10	22	21	16	18
<i>dl</i> -Isoserine	"	4	-1	-7	-5	0	-1	0

oxygen uptake or ammonia production observed. The deaminase of the tissue apparently acts only upon α -amino groups.

The *dl*- α , β -diaminopropionic acid was readily attacked by both the liver and kidney tissues but the oxygen uptakes observed were less than one-half of the values which would be expected for the deamination of the α -amino group of the *d* isomer. Although there is, at present, no direct proof that the deamination observed was the removal of the α -amino group of this amino acid, the comparison of the results with those obtained with β -alanine and with isoserine indicates that the β -amino group was

probably not attacked. The lower values for the respiration and ammonia production observed with this compound suggest that the introduction of an amino group into the β position of the alanine molecule inhibits, but does not completely abolish the deamination at the α position. A similar, but less marked effect, was observed with the *dl*-serine when the β substituent is a hydroxyl group. The serine was attacked more readily by kidney tissue than was the diamino derivative, but was less readily decomposed than was the unsubstituted alanine. With liver tissue, the diamino derivative was apparently more easily decomposed than was the serine.

Because of the differences in the deaminizing activity of the slices from the different animals, the data for each amino acid show considerable variation. As has been pointed out, direct comparisons should be made only between the data which were obtained with the tissues from the same animal. As a summary of the various experiments, however, the maximum, minimum, and average per cent of decomposition of each of the amino acids studied is presented in Table III. It is readily seen that the β -alanine and the *dl*-isoserine were not oxidized and that the average values for the *dl*-serine and *dl*- α,β -diaminopropionic acid were less than the minimum values found with the control compound, *dl*-alanine.

The authors wish to express their appreciation to Professor Howard B. Lewis for his interest and advice in the conduct of the experiments.

SUMMARY

The relationship of the chemical structure of the substrate to the activity of the *d*-amino acid deaminase of rat kidney and liver has been investigated. *dl*-Serine and *dl*- α,β -diaminopropionic acid were less readily oxidized than was the control amino acid, *dl*-alanine. *dl*-Isoserine and β -alanine were not attacked. The results indicate that the *d*-amino acid deaminase of rat tissues is a specific enzyme for the oxidative removal of α -amino groups, and that the substitution of a hydroxyl or an amino group in the β position suppresses but does not completely abolish the oxidative deamination at the α position.

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